d-Thyroxine Reduces Lipoprotein(a) Serum Concentration in Dialysis Patients

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Abstract. Uremia raises lipoprotein(a) (Lp(a)) serum concentration and the risk of arteriosclerosis in dialysis patients. The treatment of high Lp(a) levels is not satisfactory today. The decrease of Lp(a) in hypothyroid patients on L-T4 therapy raised the question of whether dextro-thyroxine (d-thyroxine) reduces not only serum cholesterol, but also Lp(a) serum concentration. In a single-blind placebo-controlled study, the influence of d-thyroxine therapy on Lp(a) serum concentration was evaluated in 30 hemodialysis patients with elevated Lp(a) serum levels. Lp(a) was quantified in parallel by two methods, i.e., rocket immunoelectrophoresis and nephelometry, and apo(a) isoforms were determined by a sensitive immunoblotting technique. Regardless of the apo(a) isoforms, 6 mg/d

Arteriosclerosis is accelerated in patients with end-stage renal disease (ESRD) (1). Various risk factors may be involved in the pathogenesis of arteriosclerosis in ESRD patients, such as hypertension, impaired glucose tolerance, decreased HDL, and increased serum levels of triglycerides and also of lipoprotein(a) (Lp(a)). During the past 10 yr, Lp(a) has been recognized as an independent risk factor for coronary, cerebral, and peripheral arteriosclerosis in the healthy population and also in dialysis patients. In a prospective study, Cressman and colleagues noted a significantly higher plasma level of Lp(a) in dialysis patients with complications due to arteriosclerosis compared with patients without such complications (2).

Today, treatment of elevated Lp(a) plasma concentrations is not satisfactory. A low-cholesterol diet (3), cholestyramine (4), and (β)hydroxy-((β)methyl glutaryl-CoA reductase inhibitors (5–7) did not reduce Lp(a) plasma levels. Niacin therapy was followed by a significant decrease of Lp(a) levels, but was poorly tolerated due to its side effects (8). Gemfibrozil (2 × 600 mg) reduced Lp(a) levels by 27% in hypercholesterolemic patients (9). N-Acetylcysteine also mildly reduced plasma levels of Lp(a) (10). Furthermore, sex hormones may influence Lp(a) plasma concentrations. The use of estrogen as contraceptive (11), as postmenopausal hormone therapy (12), or for treatment of men with prostatic cancer was followed by a decrease of serum Lp(a) levels (13). Recently, lifibrotil, a new lipid-lowering agent, has also been shown to lower Lp(a) concentrations by approximately 30% (14).

Recent clinical observations indicated that treatment with the thyroid hormones levo-triiodothyronine (L-T3) or levo-thyroxine (L-T4) reduces cholesterol concentrations and also Lp(a) levels in hypothyroid patients (15–18). This raised the question of whether dextro-thyroxine (d-thyroxine), which does not have significant general metabolic effects as l-thyroxine, but does lower serum cholesterol levels, is able to reduce Lp(a) serum concentrations. Therefore, we studied the effect of d-thyroxine on serum lipids and Lp(a) in dialysis patients with elevated Lp(a) serum concentrations.

Materials and Methods

Patients

A total of 39 (13 women, 26 men) chronic hemodialysis patients with a basal Lp(a) concentration of more than 250 mg/L were enrolled in the study. The mean age was 58.8 ± 11.0 yr (mean ± SD), and the duration of dialysis therapy was 71.2 ± 79.7 mo. All of the patients were routinely dialyzed for 5 h 3 times a week using Gambro AK10 (Gambro, Lund, Sweden) or Fresenius MTS 2008 C or 4008 C machines (Fresenius, Bad Homburg, Germany), and GFS15 M, GFS 15H (Gambro, Hechingen, Germany), F6, F60, F50 dialyzers (Fresenius) or Renal 15U dialyzers (Kawasaki, Shmagawa KU, Tokyo,
Japan), with a dialysate flow of 500 ml/min and a blood flow of 240 to 300 ml/min with a double-needle technique. The dialysis devices and procedure were kept constant throughout the study period, and urea reduction rates were consistently >60% and varied only by ±3% in the single patient. The underlying renal diseases were glomerulonephritis (n = 15), analgesic nephropathy (n = 4), diabetic nephropathy (n = 6), obstructive nephropathy (n = 2), amyloidosis (n = 1), tuberculosis (n = 1), polycystic kidney disease (n = 3), nephroclerosis (n = 3), and unknown (n = 4). Euthyroidism was documented by the serum concentrations of thyroid-stimulating hormone (TSH), total thyroxine, and triiodothyronine in all patients during the placebo period preceding the D-thyroxine treatment. Median values (and ranges) were within the normal range: TSH, 1.24 (0.02 to 3.0) mU/L; total thyroxine, 77.1 (45 to 132) nmol/L; and total triiodothyronine, 1.75 (1.32 to 2.67) nmol/L. Medication of patients included subcutaneous or intravenous recombinant human erythropoietin, intravenous iron gluconate, and orally vitamins, calcium salts, or aluminum-containing phosphate binders, sedatives, digitals, nitrates, and anti-diabetics, but no antilipid therapy other than D-thyroxine. The treatment was kept constant except for minor modifications, which have no known effect on lipid metabolism. Twelve of the 30 evaluated patients were continuously treated with antihypertensive drugs. In only one patient was metoprolol treatment (1 × 50 mg) stopped after 5 wk of the D-thyroxine treatment.

**Study Drug Therapy**

In this randomized single-blind study, the patients were subdivided into two groups: control subjects (n = 13) and the D-T4 group (n = 26). The D-T4 group was further subdivided into D-T4 group 1 (n = 15), with a placebo period of 4 wk, and D-T4 group 2 (n = 11), with a placebo period of 8 wk, before D-thyroxine treatment was started. D-Thyroxine (Dynothel®, Fa. Henning, Berlin, Germany) treatment was started with 2 mg/d, increased every 4 wk by 2 mg to a maximum of 6 mg/d. The daily dosage of the medication was organized in separate pillbox compartments to ensure correct and timely administration of the medication. The D-thyroxine therapy was followed by a 12-wk washout period without further medication. Nine patients were excluded from the final evaluation: three control subjects for relapse of vasculitis, decompensating diabetes, or transfer to another dialysis center; three patients in the D-T4 group 1 for septicemia, tuberculosis, or noncompliance; and three patients in the D-T4 group 2 due to occlusive bowel disease or obvious noncompliance, and one patient who refused to continue the study because of itching during the placebo phase.

During the 32 wk of the study, laboratory tests were performed before and at 4-wk intervals during the phases of placebo and D-thyroxine treatment. During the final washout period, measurements were done after 6 and 12 wk. The study was conducted in accordance with the guidelines proposed in the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Medical Center of the University of Heidelberg, and written informed consent was obtained from the patients.

**Assessment of Efficacy**

Blood was taken just before a hemodialysis session. Serum and plasma (heparinized) were refrigerated at 4°C or frozen at −21°C. All measurements were done within the next week except for the determination of T3, T4, and TSH, which were measured simultaneously at the end of the study after storage at −21°C. The quantification method of thyroxine and triiodothyronine did not differentiate between the dextro and levo isomers.

Lp(a) was measured by two different methods, rocket electrophoresis and nephelometry, in two different laboratories in Heidelberg and Freiburg. During the electroimmunodiffusion test (Immuno AG, Vienna Austria), Lp(a) formed a rocket-like precipitation in the anti-apo(a) antibody-loaded agarose gel. The length of that rocket-like precipitation corresponds to the concentration of the Lp(a). Nephelometrically, Lp(a) was determined with polyclonal antibodies (Inkstar, Stillwater, MN) on a Behring Nephelometer (Behring, Marburg, Germany) as described in detail by Nauck et al. (19). apo(a) isoform determination was performed on whole serum, using a sensitive immunoblotting technique described previously (20) and modified as follows. Briefly, 12 µl of serum was mixed with 100 µl of sample buffer containing 5% sodium dodecyl sulfate, 2% mercaptoethanol, and 2% bromphenol blue. After 10 min of boiling in a water bath, the reduced samples were applied to a 4% polyacrylamide electrophoresis gel and run for approximately 2 h at 30 mA. After electrotransfer of the proteins to Immobilon polyvinylidifluoride transfer membranes (Millipore, Bedford, MA), membranes were blocked with 3% bovine serum albumin, incubated with a polyclonal anti-apo(a) antibody (Immuno AG), and detected with the anti-sheep IgG (Fc) alkaline phosphatase method. A standard of a defined isoform pool (B, S1, S3, S4, >S4) was used on each gel.

The functional thrombin-coagulable fibrinogen was quantified with the Clauss method (Biomatic Sarstedt, Nümbrecht, Germany). Total cholesterol and triglycerides were measured enzymatically (Boehringer, Mannheim, Germany). VLDL cholesterol (VLDL-C), LDL cholesterol (LDL-C), and HDL cholesterol (HDL-C) were analyzed with a combined ultracentrifugation and precipitation technique (21). The "pure" LDL cholesterol, corrected for the content of Lp(a) in the LDL, was calculated as: "pure" LDL = LDL-C − 0.3 × Lp(a) (22).

Aspartate aminotransferase, alanine aminotransferase, urea, creatinine, and uric acid were measured by the Chem-1 chemistry analyzer (Bayer Technicon, Munich, Germany). C-reactive protein was measured by rate nephelometry with the Array 360 analyzer (Beckman, Munich, Germany).

**Statistical Analysis**

Mean values and SEM (±SD) or median values and ranges are given unless otherwise stated. In the control group and the total group of D-thyroxine-treated patients, the changes of serum concentration of lipids and other parameters during the study period were analyzed by the Wilcoxon matched-pairs signed rank test, if the Friedman two-way ANOVA was significant.

In Caucasian populations, serum Lp(a) concentrations show no Gaussian distribution but do show a skewed distribution. In the Kolmogorov–Smirnov test of normal distribution, normal Gaussian distribution was also not given in all other laboratory data, possibly due to the limited number of control subjects and D-thyroxine-treated patients. Therefore, the individual serum concentrations of Lp(a) and other variables assessed during the D-thyroxine treatment period and washout period were compared with the mean of the two concentrations found during the preceding placebo period. By this method, the percent changes were calculated for each single laboratory result and analyzed by the Kruskal–Wallis ANOVA by ranks. Furthermore, at different points in time, the percent changes of lipids and other laboratory results in D-thyroxine-treated patients were compared with those in control patients by the Wilcoxon rank sum test.

**Results**

As measured by electroimmunodiffusion test, mean Lp(a) decreased significantly from 47.4 ± 22.9 to 34.2 ± 16.6 mg/dL...
under 6 mg/d D-thyroxine therapy (Table 1, Figure 1). This decrease by 27 ± 13% is significant compared with the control patients (P < 0.01). A relevant change of the Lp(a) levels was not found in the control group (52.2 ± 28.3 mg/dl at the beginning, and 48.3 ± 30.6 mg/dl (−9.9% ± 8.4%) after 16 wk of the treatment period. During the next washout period, Lp(a) serum concentrations reincresed and were similar to those found in control patients.

When Lp(a) was measured by nephelometry, D-thyroxine therapy reduced mean Lp(a) levels from 46.9 ± 21.2 to 38.7 ± 23.3 mg/dl after week 12 and to 41.2 ± 22.6 mg/L after week 16 of therapy (Table 1). This is a significant decrease of 20.3 ± 17.1 and 13 ± 17%, respectively (P < 0.05), compared with the control patients in whom Lp(a) levels did not change significantly (52.5 ± 31.6 mg/dl before and 49.3 ± 33.3 mg/dl after week 12, and 57.3 ± 41.9 mg/L after week 16 of the treatment period). After the final washout period, Lp(a) levels were not different in previously D-thyroxine-treated and control patients.

In parallel, total cholesterol levels decreased from 192.0 ± 37.9 to 161.1 ± 38 mg/dl (16.3 ± 10.5%, P < 0.01) (Figure 2) during maximal D-thyroxine therapy and remained stable in the control subjects (180.7 ± 33.3 mg/dl versus 183.6 ± 38.02 mg/dl) (Table 1). LDL cholesterol remained stable in the control subjects and decreased from 128.8 ± 33.1 mg/dl to 101 ± 22.8 mg/dl (20.7 ± 10.4%, P < 0.01) (Figure 3) during D-thyroxine therapy. The “pure” LDL was constant in the control group but decreased in the D-thyroxine-treated patients, from 114.5 ± 35.5 to 90.3 ± 24.7 (P < 0.01) (Table 1). HDL cholesterol levels did not change markedly in the control patients (25.4 ± 4.3 mg/dl versus 26.0 ± 3.1 mg/dl) or D-thyroxine-treated patients (36.2 ± 14.1 mg/dl versus 37.7 ± 15.2 mg/dl). Furthermore, the LDL cholesterol/HDL cholesterol ratio did not change in the control patients (4.7 ± 1.4 versus 4.5 ± 1.2) but decreased significantly in the D-thyroxine-treated patients, from 4.0 ± 1.6 to 2.9 ± 1.0 (23 ± 14.5%, P < 0.01). The VLDL cholesterol also decreased under D-thyroxine therapy, from 27.8 ± 15.4 to 23.2 ± 16.3 mg/dl (19.8 ± 42.2%, P < 0.05). The decrease of serum triglyceride levels (from 166 ± 76 to 138.5 ± 69.3 mg/dl) agreed with the fall of VLDL cholesterol levels, but was not significant.
**Lp(a) Rocket Electrophoresis**

*Figure 1.* Changes of lipoprotein(a) (Lp(a)) serum concentration expressed as percentage of the mean Lp(a) level during the preceding placebo period in the individual patients (mean ± SEM). Control subjects (●), D-T4 group 1 (■), D-T4 group 2 (▲), and all D-T4-treated patients (□). Significant difference of the percent changes between control subjects and all D-T4 patients at the different points of time. *P < 0.05; **P < 0.01 (Wilcoxon rank sum test).

**Cholesterol**

*Figure 2.* Changes of total serum cholesterol concentration expressed as percentage of the mean total cholesterol level during the preceding placebo period in the individual patients (mean ± SEM). For symbols and significance, see Figure 1.

D-Thyroxine therapy increased serum concentrations of T4 from 90.5 ± 25.5 to 147.5 ± 40.8 ng/ml (*P < 0.001) and of T3 from 1.8 ± 0.4 to 4.1 ± 1.2 ng/ml (*P < 0.001) and decreased TSH serum concentrations from 1.1 ± 0.8 mU/L to <0.01 mU/L (*P < 0.001). All other variables, including C-reactive protein, albumin, total protein, and fibrinogen concentration, remained unaffected in control and D-thyroxine-treated patients throughout the study period.

Body weight (Table 1) or predialytic pulse rate and BP did not change markedly. Other clinical symptoms of hyperthyroidism were not found in any of the patients in response to the D-thyroxine therapy. During the preceding placebo period, one patient reported mild paroxysmal tachycardia, with heart rates up to 100 beats/min for 6 d, which stopped spontaneously.

**Discussion**

In agreement with previous reports, D-thyroxine therapy reduced serum levels of total cholesterol, LDL cholesterol, and, to a lesser extent, VLDL cholesterol (23,24). The decrease of LDL cholesterol was also evident if the LDL cholesterol was
corrected for the Lp(a) content in the LDL fraction ("pure" LDL cholesterol). In the present study, d-thyroxine therapy with a maximum dose of 6 mg/d reduced elevated Lp(a) levels in uremic patients, and Lp(a) reincreased during the washout period after d-thyroxine treatment. Progressing renal failure is commonly accompanied by an increase of the Lp(a) serum concentration. Some reports indicated that, in contrast to other factors such as growth hormone (25), hypothyroidism (17,18), or low estrogen levels (12,26), uremia induces a specific increase of high molecular Lp(a) isoforms (27) that is reversed after kidney transplantation (28). In our study, d-thyroxine reduced Lp(a) in dialysis patients regardless of the molecular weight of apo(a) isoforms. Today, it is possible to differentiate 34 Lp(a) phenotypes (29). The apo(a) phenotyping method used in the current study distinguishes six apo(a) isoforms and thus breaks down the larger number of apo(a) alleles into the groups originally described by Utermann et al. (30,31). However, this is not a relevant limitation of our study, which included only 30 patients. Given the tremendous heterogenicity of the apo(a) locus, a phenotyping method with higher resolution would have revealed different phenotypes in almost all of the individuals studied. Obviously, analysis of such data would have required post hoc stratification, and the information gained by a more sophisticated phenotyping method would not have been used. When our patients were subdivided into two groups of Lp(a) phenotypes, i.e., ≤S2 (B, S1, S2, high molecular Lp(a)) and >S2 (S3, S4, >S4, low molecular Lp(a)) (27,28), the mean percent reduction of Lp(a) levels under d-thyroxine therapy was identical in both groups. Repeated determination of apo(a) phenotypes did not indicate a relevant change in size of Lp(a) in the individual patients during d-thyroxine therapy. In good agreement with these findings, antithyroid therapy was followed by a comparable increase of Lp(a) in hyperthyroid patients with high or low molecular weight apo(a) phenotypes as well (32).

The question of the optimal and most reliable method of quantifying serum concentrations of Lp(a) is under continuing discussion. In the present study, the reduction of Lp(a) was documented by two different, highly validated methods of Lp(a) quantification, performed in two separate laboratories. Thus, it is unlikely that the method of Lp(a) measurement was influenced by changes of Lp(a) structure or size during the study period.

The daily dosage of medication was organized in separate pillbox compartments, so that each patient could always be certain of taking the tablets. Furthermore, the increase of T4 and T3 serum levels, as well as the complete suppression of TSH secretion in all d-thyroxine-treated patients, indicated good compliance. Significant adverse effects such as symptoms of hyperthyroidism were not observed during d-thyroxine therapy, using a very pure d-thyroxine preparation with an L-T4 content of <0.1%.

The mechanism of Lp(a) reduction under d-thyroxine remains unknown. l-Thyroxine or l-triiodothyronine seems to induce a higher expression and activity of LDL receptors followed by enhanced LDL cholesterol catabolism (33). In cultured fibroblasts, T3 increases the number of LDL receptors and the degradation rate of LDL cholesterol (34). A determinant role of LDL receptors in the catabolism of Lp(a) has been postulated, but is not proven. The Lp(a) kinetics after LDL apheresis (35) and the fractional catabolic rate of Lp(a) in subjects with familial hypercholesterolemia suggest that in vivo Lp(a) is not markedly cleared by an LDL receptor-mediated mechanism.

The rather constant Lp(a) serum concentration under HMG-CoA reductase inhibitor therapy followed by upregulation of
LDL receptors and decrease of LDL serum concentration also points to some differences in the metabolism of LDL and Lp(a). In our patients, thyroxine therapy reduced both Lp(a) and LDL cholesterol by an average of approximately 20%. The individual decreases of Lp(a) and LDL cholesterol did not correlate significantly but varied markedly from patient to patient.

In patients exhibiting the same apo(a) isofrom, different production rates of apo(a) may be more important for the serum levels of Lp(a) than different rates of Lp(a) catabolism (36). In patients with nephrotic syndrome, Lp(a) serum concentration is increased, but normalized when proteinuria disappeared (37). In patients with liver diseases, plasma levels of apo(a) significantly correlated with the microsomal function and synthetic capacity of the liver, as indicated by the levels of serum albumin and coagulation factor activities. In rats, triiodothyronine may suppress apo B messenger RNA (38). De Bruin et al. postulated that thyroid hormones directly suppress synthesis and secretion of the apo(a) moiety of Lp(a) in humans and animals (18). Additional studies are needed to clarify the mechanism of decreasing plasma levels of Lp(a) under L-T4 and thyroxine therapy.

Serum levels of l-triiodothyronine and l-thyroxine tend to be lower in dialysis patients compared with healthy control subjects, and in some patients low T3 syndrome is found (39–41). In the present study, however, hypothyroidism can be excluded by normal TSH serum levels. Furthermore, a significant effect of l-thyroxine is unlikely. The d-thyroxine preparation used contains <0.1% l-isofroms. Therefore, the maximal dose of 6 mg of d-thyroxine included <6 µg of L-T4 and a very small amount of L-T3. Any significant metabolic effect cannot be attributed to the small amounts of L-T4 and L-T3. Similar doses of that d-thyroxine preparation were used in patients with hyperthyroidism due to pituitary resistance to thyroid hormones. In such patients, d-thyroxine suppresses TSH secretion, and the symptoms of hyperthyroidism disappear (42). Hamon et al. treated hypothyroid children after total thyroidectomy plus radioiodine ablation for thyroid cancer 10 d with 24 mg of d-thyroxine per day. The cholesterol levels decreased markedly, and the authors recommended that “the ban on d-thyroxine could be reevaluated since purer preparations are available” (43).

In conclusion, the study presented here showed that d-thyroxine can significantly reduce serum concentration of Lp(a). Additional studies are required to prove the long-term effect of d-thyroxine therapy in dialysis patients and the effect in nondialysis patients, and to evaluate the benefits of lowering the Lp(a) serum concentration, such as reduced risk of arteriosclerosis in patients with elevated Lp(a). The clarification of the mechanism by which d-thyroxine reduces Lp(a) levels may help to determine combinations of drugs with different mechanisms that lower Lp(a) levels more effectively.

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